USAFSAM-TR-89-1

AD-A221 893

MECHANISM OF INTERACTION OF RADIOWAVES AND MICROWAVES WITH DEOXYRIBONUCLEIC ACID (DNA)

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March 1990

Final Report for Period April 1985 - July 1988

Prepared for
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NOTICES

This final report was submitted by the Physics Department, King's College London, Strand, London WC2R 2LS, United Kingdom, under contract AFOSR-85-0183, job order 2312-W1-07, with the USAF School of Aerospace Medicine, Human Systems Division, AFSC, Brooks Air Force Base, Texas. Dr. Johnathan L. Kiel (USAFSAM/RZP) was the Laboratory Project Scientist-in-Charge.

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The Office of Public Affairs has reviewed this report, and it is releasable to the National Technical Information Service, where it will be available to the general public, including foreign nationals.

This report has been reviewed and is approved for publication.

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UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE

REPORT DOCUMENTATION PAGE

Form Approved OMB No. 0704-0188

1a. REPORT SECURITY CLASSIFICATION Unclassified	1b RESTRICTIVE MARKINGS
2a. SECURITY CLASSIFICATION AUTHORITY	3 DISTRIBUTION/AVAILABILITY OF REPORT Approved for public release; distribution is unlimited.
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE	
4. PERFORMING ORGANIZATION REPORT NUMBER(S)	5 MONITORING ORGANIZATION REPORT NUMBER(S) USAFSAM-TR-89-1
6a. NAME OF PERFORMING ORGANIZATION Physics Department King's College London 6b. OFFICE SYMBOL (If applicable)	7a. NAME OF MONITORING ORGANIZATION European Office of Aerospace Research and Development (LSB)
6c. ADDRESS (City, State, and ZIP Code) Strand, London WC2R 2LS, UK	7b ADDRESS (City, State, and ZIP Code) 223-231 Old Marylebone Road London NW1 5TH, UK
8a. NAME OF FUNDING/SPONSORING ORGANIZATION USAF School of Aerospace Medicine 8b OFFICE SYMBOL (If applicable) USAFSAM/RZP	9 PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER Grant AFOSR-85-0183
8c. ADDRESS (City, State, and ZIP Code) Human Systems Division (AFSC) Brooks Air Force Base, TX 78235-5301	10 SOURCE OF FUNDING NUMBERS PROGRAM ELEMENT NO: 61102F; PROJECT NO: 2312; TASK NO: W1; WORK UNIT ACCESSION NO: 07

11. TITLE (Include Security Classification)

Mechanism of Interaction of Radiowaves and Microwaves with Deoxyribonucleic Acid (DNA)

12. PERSONAL AUTHOR(S)
Grant, E.H.

13a. TYPE OF REPORT	13b TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Final	FROM 85/04/01 TO 88/07/31	1990, March	33

16. SUPPLEMENTARY NOTATION

17. COSATI CODES			18 SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
FIELD	GROUP	SUB-GROUP	Microwave resonance, Dielectric loss, Microwave attenuation, Deoxyribonucleic acid (DNA), Permittivity
06	07		
20	03		

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

Measurements have been made of the relative permittivity and dielectric loss of aqueous solutions of DNA in the frequency range 1 - 10 GHz. The four types of DNA studied were plasmid, human, xenopus, and calf thymus. No evidence of resonance absorption or enhanced absorption was evident from the behavior of the dielectric data.

Extension of the dielectric measurements down to frequencies of a few Hz revealed the existence of dispersion regions caused by ionic motion. (keywords:)

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT	21 ABSTRACT SECURITY CLASSIFICATION
☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT ☐ DTIC USERS	Unclassified
22a. NAME OF RESPONSIBLE INDIVIDUAL Johnathan Kiel	22b. TELEPHONE (Include Area Code) (512) 536-3583 22c. OFFICE SYMBOL USAFSAM/RZP

DD Form 1473, JUN 86 *Previous editions are obsolete.* SECURITY CLASSIFICATION OF THIS PAGE

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INTRODUCTION

The overall objective of this research was to gain a full understanding of how radiowaves and microwaves interact with aqueous solutions of deoxyribonucleic acid (DNA). The program was divided into 2 parts: the first concerned with dielectric measurements at microwave frequencies where the aqueous component was dispersing; and the second, with measurements at radiofrequencies where the contribution to the frequency dependent dielectric properties was through the DNA molecule. The microwave work showed that the dielectric behaviour of the various types of DNA studied was purely classical. There was no evidence of resonance phenomena and no indication of enhanced absorption of any kind in the frequency range studied (1-10 GHz). This work is described in full in this report.

Although the original program was scheduled as a 3-year project, a fourth year extension was sought, and approved, to enable the work at radiofrequencies to be completed. Consequently, in the present report, the description of this part of the program is restricted to a summary of the progress made to date (in Appendix C: Supplement), plus a full and comprehensive description of the work next year.

BACKGROUND

A considerable amount of experimental work on the dielectric properties of aqueous solutions of DNA has been carried out over the past decade (1-8). Much of the research has concluded (1-6) that the dielectric behaviour of DNA in water at radiowave and microwave frequencies can be described by classical dielectric theory, based on the Debye equations or by some small deviation therefrom, such as the Cole-Cole function. An example of how these equations can be used for this purpose has been described by us previously (4).

In contrast, other workers have claimed (7,8), on the basis of their measurements, that the dielectric behaviour of DNA in aqueous solution exhibits an unusually high attenuation coefficient over a wide frequency range, which cannot be accounted for by any of the traditional dielectric theories. Moreover, for one particular type

of DNA (plasmid), resonance absorption behaviour has been reported (8,9) as occurring at frequencies of a few GHz, an observation which is quite unprecedented for experimental studies of the dielectric behaviour of biological molecules in solution. This experimental work, by Edwards, Davis, Saffer and Swicord (8), has been supported by the theoretical calculations of Van Zandt, Prohofsky, and their colleagues (10,11), who developed a model of electromagnetic energy coupling through acoustical vibrations along the axis of helical polymers. An alternative approach which would also account for the experimental DNA data is that of Scott (12), who applied a non-linear soliton approach by assuming the excited acoustic wave to be anharmonic.

The conclusions drawn by Edwards et al.(8) from their experimental observations have not, however, been universally accepted. Therefore, one of the purposes of the present work was to measure independently the dielectric properties (relative permittivity ε' and dielectric loss ε'') of aqueous solutions of DNA prepared by exactly the same procedure as that adopted by Edwards et al.(8).

In addition to their work on resonances, the same research group have observed (in the same experimental program) a considerable increase in the microwave absorption occurring in DNA solutions after the addition of the enzyme Deoxyribonuclease 1 (DNase 1) (8). In the present work, the decision was therefore made to explore this possibility by using 3 types of DNA: xenopus erythrocyte, human Y chromosome, and calf thymus.

Prior to the present program, no dielectric measurements had been carried out on circular DNA at radiofrequencies, although some RF work had been done on (linear) calf thymus DNA -- these measurements being made at room temperature (1,2). Hence a further decision was made to extend the microwave measurements on plasmid DNA down to lower frequencies and temperatures, in order to characterize the nature of the interaction in this part of the frequency spectrum. To perform worthwhile measurements on the frozen material, a relatively high concentration of DNA is required; and this condition cannot be met in the case of plasmid DNA. For purified commercial calf thymus DNA, however, less of a problem is encountered in preparing a 1% solution, which is the minimum concentration needed to give useful data. Such samples were therefore investigated in the present program at frequencies of 1 kHz - 10 MHz, and at temperatures down to -50 °C (-58 °F). Previous work by us on ocular tissues (13) has shown that dielectric measurements on frozen biological material allow dispersive processes within the water to be studied without the interference of mechanisms of polarization arising from the biological macromolecules. Since the structural integrity of DNA and similar molecules depends upon the properties of the associated water (14,15), characterizing these properties as accurately as possible is important, and a dielectric study is one means of

achieving this aim. Another technique, in which we were also involved, is that of Thermal Depolarization (refer to Appendix C: Supplement).

MATERIALS AND METHODS

Preparation of Samples

For the enzyme-related studies, 3 forms of DNA were prepared: DNA from xenopus erythrocytes of molecular weight 30 M, which corresponds to 45 kbp (kilo base pairs); DNA from human Y chromosomes of molecular weight about 100 kbp, and Sigma Type 1 highly polymerized DNA from calf thymus. Samples were purified to give protein-free samples, which were then dissolved in buffer.

With respect to the plasmid DNA, 3 forms were studied: pUC8.c1 (supercoiled); pUC8.c2 (supercoiled); and pUC8.c2 (relaxed). Plasmid pUC8.c1 is a small, covalently closed, circular 2.7 kb double-stranded DNA molecule. Most of the plasmid DNA isolated from *E. coli*, the host organism, is in the form of supercoiled molecules that have superhelical twists.

Plasmid DNA was obtained from *E. coli* strain HB 101, grown in tryptone yeast broth supplemented with glycerol overnight at 37 °C (99 °F). Harvested cells were lysed by incubation in 50 mM Tris, 50 mM EDTA, 25% (w/v) sucrose pH 8.0 containing lysozyme (2 mg/ml) at room temperature for 10 min, followed by addition of 1 volume of 0.3% triton X-100 in 187.5 mM EDTA, 150 mM Tris pH 8.0. After centrifugation at 70,000 g for 60 min, the supernatant was diluted 1:2 and digested with RNAase A (0.6 mg/ml) for 10 min at room temperature. The sample was extracted 3 times with 1 volume of phenol:chloroform:isoamyl alcohol (25:24:1), and once with chloroform:isoamyl alcohol (25:1). Plasmid DNA was precipitated by addition of 0.1 volume of 3 M sodium acetate pH 6.5 and 2 volumes cold ethanol. After 12 h at -20 °C (-4 °F), DNA was recovered by centrifugation for 15 min at 12,000 g. The pellet was redissolved in 50 mM Tris, 10 mM EDTA, 0.5 M NaCl pH 7.5, and loaded on a column (1 x 25 cm) of Sepharose 4B that was washed with the same buffer. Next, 1-ml fractions were collected and the absorbance at 2260 nm was monitored. The first peak, corresponding to plasmid DNA, was retained. Plasmid DNA was extracted initially with phenol/chloroform/isoamyl alcohol, and next with chloroform/isoamyl alcohol, and was then reprecipitated with ethanol.

Residual ethanol was removed under vacuum, and the pellet was resuspended in storage buffer (10 mM Tris, 10 mM NaCl, 1 mM EDTA pH 7.5). Only DNA samples with a ratio A_{260}/A_{280} superior to 1.9 were

used. The purity of the plasmid form was checked by agarose gel electrophoresis. pUC8.c2 is a dimer of pUC8.c1, consisting of 2 molecules of pUC8.c1 linked to form a circular double-stranded molecule of 5.4 kb. Isolated by the method described for pUC8.c1 in the superhelical form, puC8.c2 was converted to the relaxed form by incubation with topoisomerase I, an enzyme that cuts one DNA strand, thus causing the plasmid to unwind, lose its superhelical twists, and adopt an open circular or relaxed structure. The change in form from supercoiled to relaxed is detectable by a change in electrophoretic mobility.

Topoisomerase I (from Bethesda Research Laboratories Ltd.) was used according to the suppliers' instructions. After incubation, the solution was extracted twice with phenol chloroform/ isoamyl alcohol, and DNA was reprecipitated with ethanol. The homogeneity of the relaxed form was checked by agarose gel electrophoresis.

These methods of preparation of the DNA solutions followed in detail those adopted by Edwards et al. (8).

Measurement of Permittivity

In order to provide a completely independent set of measurements to those taken at King's College, arrangements were made for the plasmid samples to be measured in the laboratory of Dr Bo Gestblom at the University of Uppsala, Sweden. Dr. Gestblom has had considerable experience in this field and is, in every way, the ideal choice as a co-worker.

The dielectric investigations that were conducted are summarized in Table 1, where the various experimental techniques are illustrated.

A common and important feature in the experimental procedure is the use of a reference sample to normalize the measured reflection and transmission coefficient. This feature will minimize systematic artifacts which may arise, for example, from slight impedance mismatches within the system. The reference liquid was pure water, the dielectric parameters of which are well known. In one case (as later discussed), the reference chosen was an aqueous electrolyte solution.

Next are given some details about the experimental measurements, as listed in Table 1; further information about the experimental set-ups can be found in the appropriate references cited.

In Time Domain Spectroscopy (TDS), the measurement is based on the study of the influence of the dielectric sample on a pulse propagating in a coaxial line. In the reflection methods, the pulse reflected from the sample r(t) is compared with the pulse reflected from the reference liquid $r_{ref}(t)$. Fourier transformation $F(\omega)=f(t)\ exp\ (-i\omega t)dt$ of both pulse shapes gives a reflection coefficient ratio $R(\omega)/R_{ref}(\omega)$ at a chosen frequency. From transmission line theory this ratio can be expressed as a function of permittivity, and solution of the corresponding equation will give the permittivity spectrum. In the transmission methods, the pulse transmitted through the dielectric sample is studied instead -- the permittivity spectrum then being worked out from the transmission coefficient ratio.

An important feature of the total transmission and total reflection TDS methods is that the time domain data will immediately give the dc conductivity σ of the sample. Thus, σ can be deduced from the ratio of the final levels of the incoming and reflected and/or transmitted pulses, $v(t\text{-->}\infty)$ and $r(t\text{-->}\infty)$ respectively. This approach makes it possible to correct directly the measured ε''_{tot} values for the conductivity contribution $\sigma/\omega\varepsilon_o$ to obtain the dipolar contribution ε''.

In measurement 1, a probe (Fig. 1) consisting of an open-ended coaxial line is placed in contact with the sample*. The signal reflected from the probe-sample interface is compared with that reflected from the probe-reference interface. The present probe, 650 mm in length, is made of 3.6 mm-diameter cable, the functional end being gold-plated and fitted with a thermocouple. The probe incorporates its own delay line, chosen to eliminate the reflection of its connector from the observational time window. Specimen holders with the DNA samples, buffer, and water are placed in a temperature-controlled unit. The samples and buffer were measured alternately by using the same water reference. The measurements of the buffer served to ensure that the technique is free from systematic experimental artifacts.

Measurement 2 consisted of an in-guide technique with a sample thickness of 0.9 mm. The specimen cell was loaded, in turn, with the DNA sample, buffer, and water reference.

The data relating to the effects of the enzyme are next presented, commencing with the values of ε' and ε'' for 0.1% solution of xenopus DNA (Fig. 2). These were obtained with a copper probe and the values indicated by (*) correspond to t=0; i.e., before the addition of the enzyme. A quantity of DNase in the presence of $MgCl_2$ was then added, and the measurements repeated at 15-min

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* *This probe was developed from a larger one which had been devised previously for measuring the permittivity and conductivity of skin (16).*

TABLE 1. DETAILS OF EXPERIMENTAL TECHNIQUES

Measurement technique	Material	Configuration	Reference
TDS(1) Reflection from a coaxial sensor	pUC8.c1 pUC8.c2 pUC8.c2 calf thymus human xenopus	supercoiled supercoiled relaxed	Gabriel et al., 1986 (16)
TDS(2) Total reflections from a 0.9-mm sample in a matched coaxial line	pUC8.c1 pUC8.c2 pUC8.c2 calf thymus	supercoiled supercoiled relaxed	Dawkins et al., 1981 (17)
TDS (3) Total transmission from a 5-mm sample in a matched coaxial line	pUC8.c2	supercoiled	Gestblom and Elmgren, 1982 (20)
TDS (4) Total transmission from a 10-mm sample in a matched coaxial line	pUC8.c2	supercoiled	Gestblom and Elmgren, 1982 (20)
TDS (5) Single reflection from a sample in a matched coaxial line	pUC8.c2	supercoiled	Gestblom and Noreland, 1984 (18)
TDS (6) Single reflection from a sample in a matched coaxial line	pUC8.c2	supercoiled	Gabriel et al., 1984 (19)
FD Total transmission from a sample in a matched coaxial line	pUC8.c2	supercoiled	Gestblom, 1982 (21)

(All measurements were made at 20 °C (68 °F)).

TDS = Time Domain Spectroscopy
FD = Frequency Domain (Spectroscopy)

intervals up to 3 h. After each measurement, a small fraction of the sample was withdrawn and EDTA added, after which the fraction was frozen and analysed by gel electrophoresis to show the state of degradation. Complete degradation occurred after 3 h.

The values (o) of ε' and ε", taken after 3-h exposure to DNase, are also shown in Figure 2, where, on first sight, the attenuation coefficient (α) might appear to have increased significantly due to enzyme action. The attenuation coefficient (α) is evaluated from ε' and ε" by:

$$\alpha = \frac{\omega}{c}\left[\frac{(\varepsilon'^2 + \varepsilon''^2)^{\frac{1}{2}} - \varepsilon'}{2}\right]^{\frac{1}{2}} \qquad \text{(Eq. 1)}$$

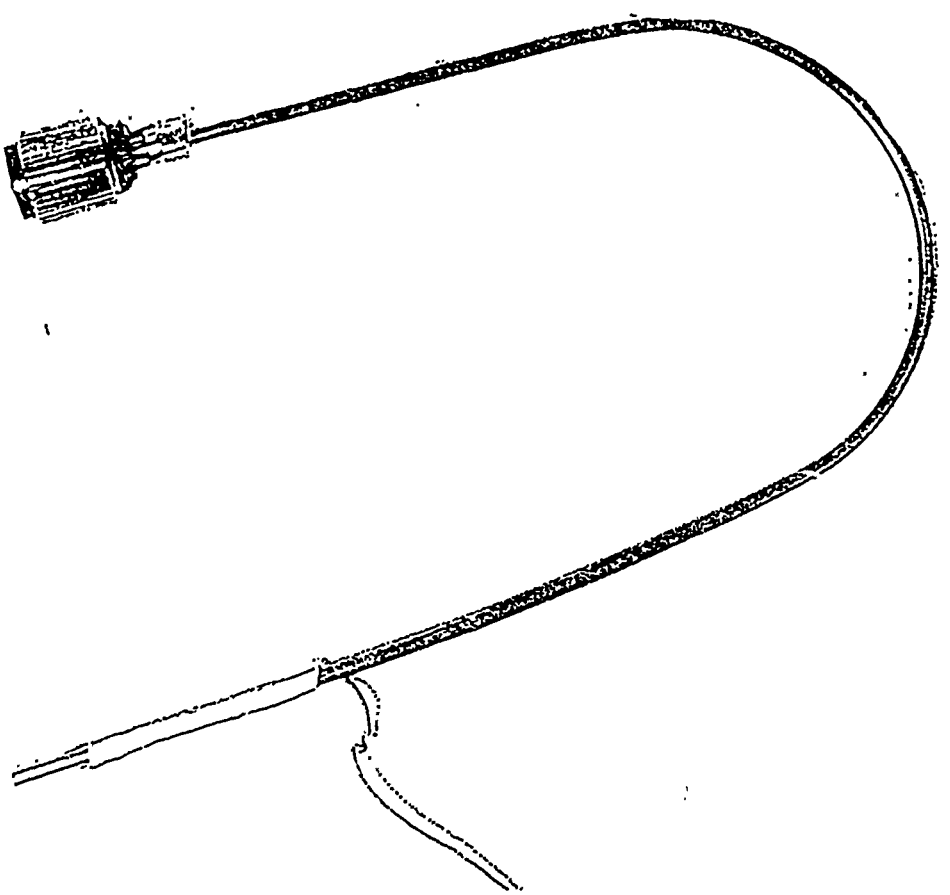

Figure 1. Coaxial probe used in measurement technique (1). (Refers to Table 1).

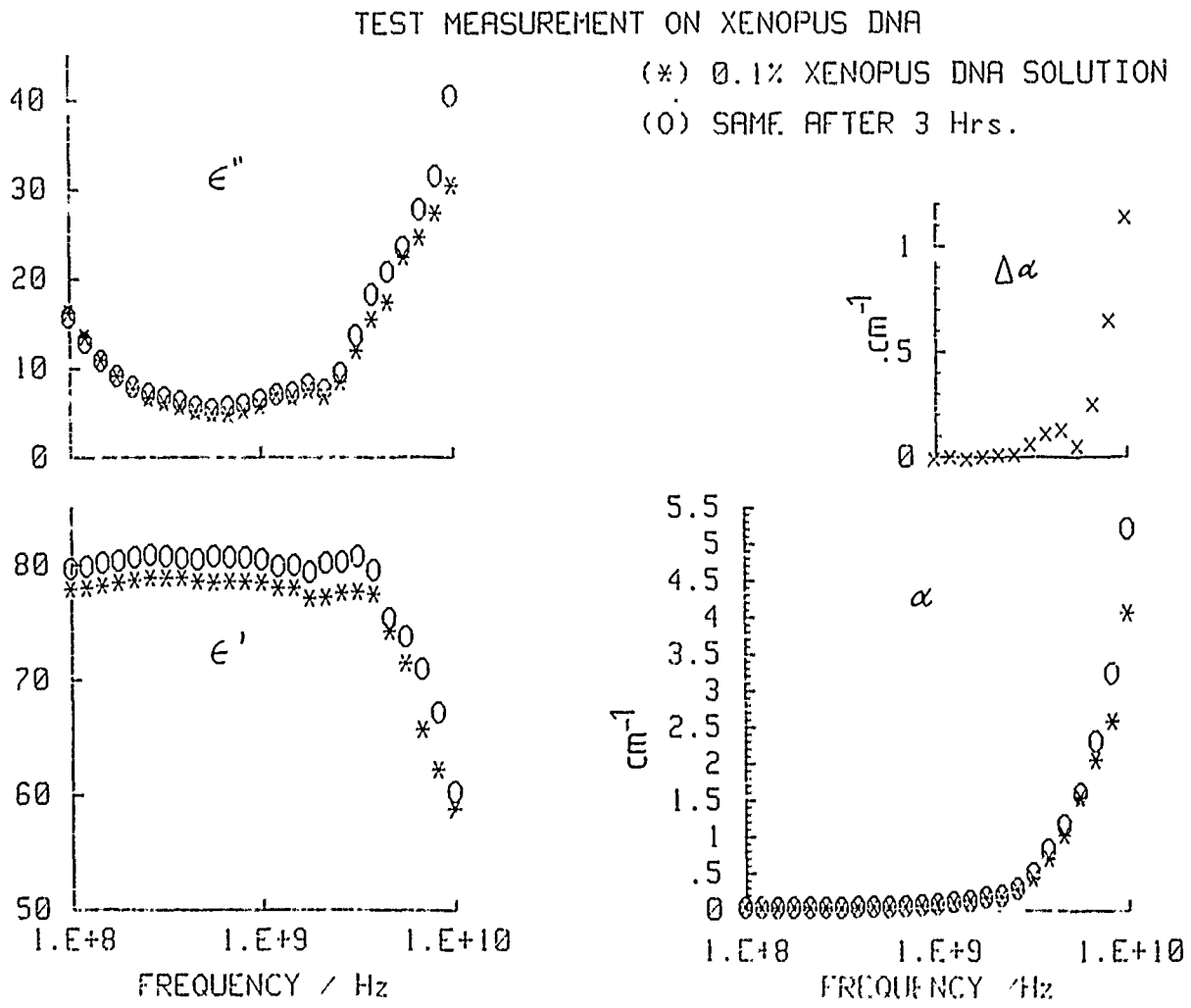

Figure 2. Measured values of ε' and ε" for xenopus DNA with a copper probe being used.

and $\Delta\alpha$ is the incremental value over the solvent. However, the probe which, at the beginning of the experiment had been perfectly flat and smooth, was found to be badly pitted because of the action of the solvent; and the consequent time-dependent malfunctioning of the probe would therefore lead to unreliable data. This hypothesis was confirmed by repeating the observation after the probe had been gold-plated, when it was observed that $\Delta\alpha=0$ (i.e., even after 3 h) the action of the DNase had no effect on the attenuation coefficient. Therefore, the conclusion may be drawn that any observation of enhanced attenuation is an experimental artifact. The experiments on the xenopus DNA were further improved by substituting water as a reference liquid by a solution (in this case, Tris buffer) having a total conductivity matching that of the DNA solution.

The experiments on the effect of DNase were then repeated using human DNA as the sample, and again employing a gold-plated probe with a matched liquid as the reference. The results are shown in Figure 3. As with the xenopus DNA, no evidence exists of any enhancement in the attenuation after 3 h of digestion with the enzyme -- the scatter of the values of $\Delta\alpha$ around the abscissa axis being entirely within the random experimental uncertainty. A similar negative effect was observed for the action of DNase on calf thymus DNA.

The experiments on xenopus, human, and calf thymus DNA were all conducted using measurement techniques 1 or 2 (Table 1). Measurement 1 was also employed as one of the seven methods for determining the dielectric behaviour of the plasmid DNA, the subject of the rest of this section. The results obtained by this method for supercoiled DNA are shown in Figure 4.* Measurement technique 2 was also employed to measure ε' and ε'' for relaxed DNA.**

With measurement technique 3, a 5-mm sample length was used. The observational time window was 10 ns. To check the measurement procedure, a dioxane-water solution was prepared and the cell was alternately filled with this test solution, the DNA solution, and pure water. The average of 36 spectra was calculated for the two solutions. The spectrum of the DNA solution is shown in Figure 5. The spectrum of the test solution is not included, but its excellent agreement with the expected behaviour of "diluted water" confirmed the reliability of the measurement procedure.

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* *The results for the relaxed DNA are included in the data in Fig. 7.*

** *The data obtained are incorporated in Fig. 7.*

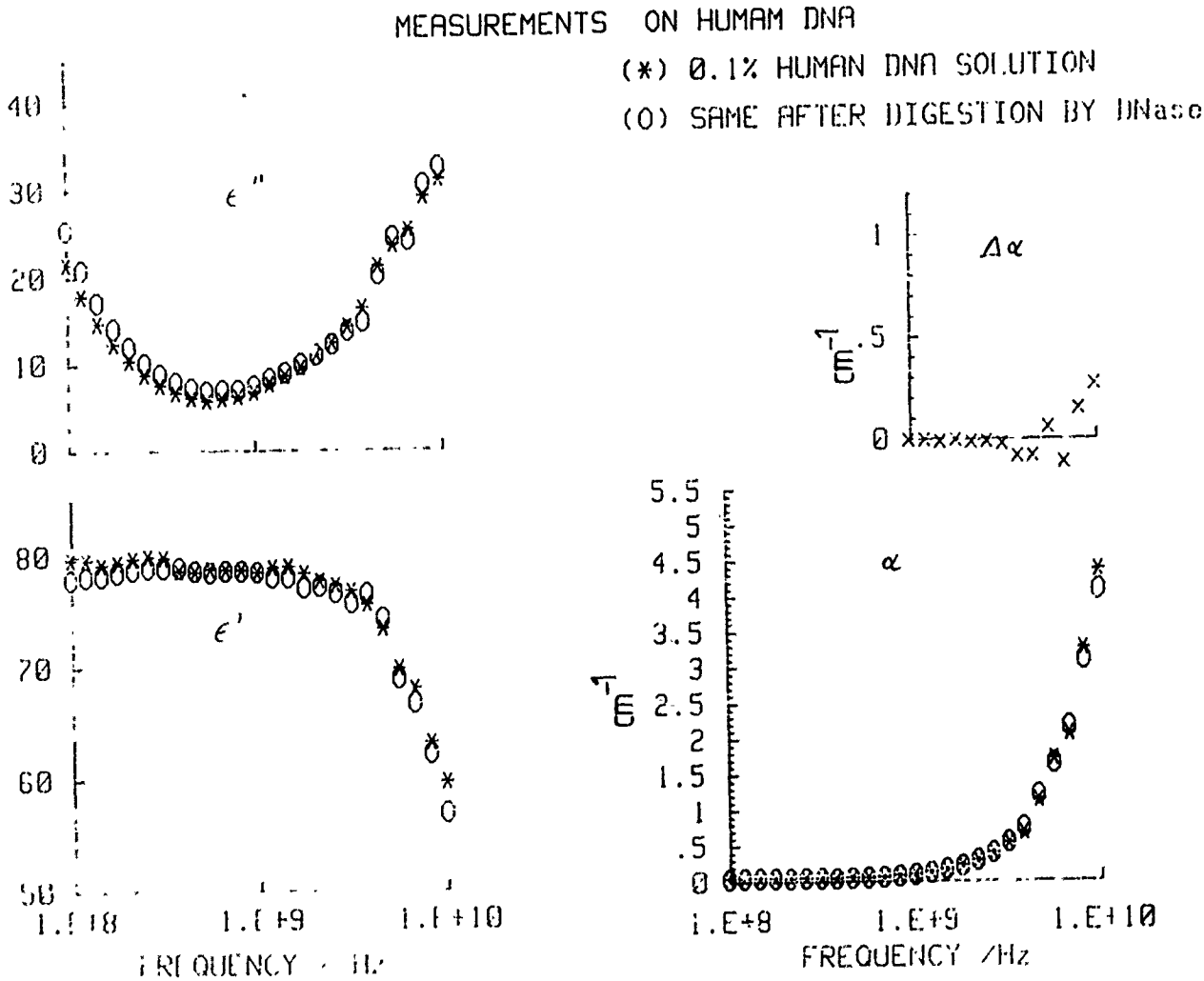


Figure 3. Relative permittivity (ε') and loss factor(ε") of human DNA before and after digestion by DNase.

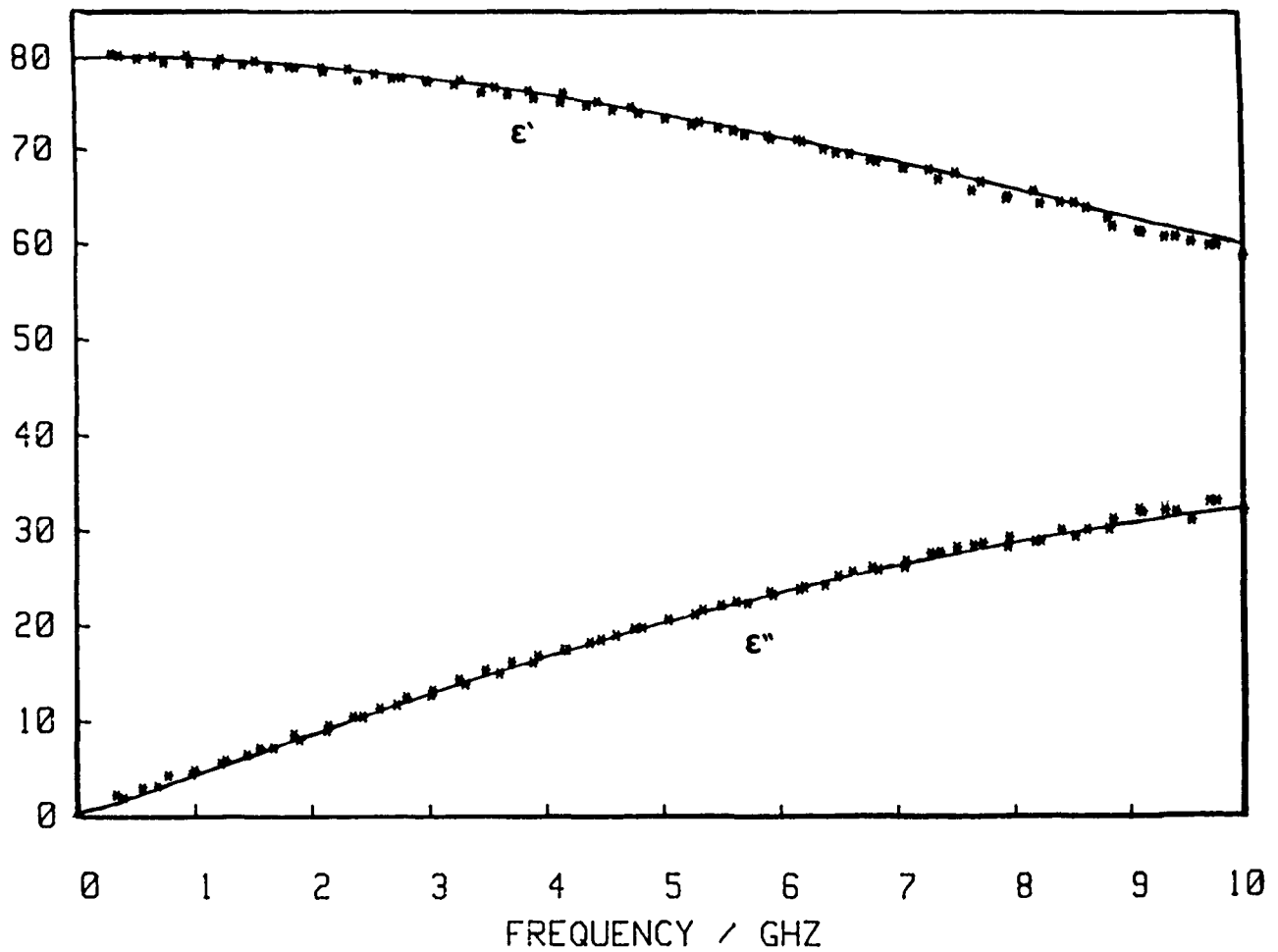


Figure 4. Relative permittivity (ε') and loss factor(ε") of 0.1% plasmid (Supercoiled) DNA solution at 20 °C. (Measurements taken at King's College London using Technique 1).

In measurement technique 4 the sample length was increased to 10 mm with a time window of 10 ns. Again, 36 independent spectra were recorded by repeated fillings of the DNA solution, the reference water, and the dioxane-water. In this case, the upper frequency limit of the spectrum was about 7 GHz, due to the strong signal absorption by the sample above this frequency.

In the single reflection method, the pulse reflected from the first air-dielectric interface of a long dielectric sample is compared with the pulse reflected from a known dielectric. In contrast to the total reflection-transmission techniques, the conductivity of the sample cannot be obtained directly from the time domain data in this case. Instead, σ has to be deduced by other methods, either independent measurements or from the $\sigma/\omega\varepsilon_o$ behaviour of ε''_{tot} at low frequencies.

In measurement technique 5 an aqueous solution of potassium chloride was used as reference. The concentration was chosen to give a standard solution with dc conductivity close to that of the DNA solution. The observational time window was 5 ns, sufficiently *long* to give accurate Fourier transforms above 1 GHz and, at the same time, sufficiently *short* to keep multiple reflected signals out of the time window. Eighteen independent spectra were recorded by repeated fillings of the DNA solution and the reference solution. The limited number of independent spectra in this case led to larger uncertainties in the dielectric parameters than for the methods already discussed.

In measurement technique 6 the sample length was 60 mm; the observational time window, 2 ns. The DNA sample and buffer were measured using water as a reference. The sample was loaded only once; the time domain data were recorded 5 times, and subsequently normalised against 5 different spectra for water.

The total transmission method can also be employed to make measurements directly in the frequency domain. In this case, the pulse generator is exchanged for an oscillator and the transmission coefficient is measured at spot frequencies, the remaining analysis being the same as for the TDS method.

In measurement technique 3, a 2-mm sample was used at 3.4 and 4.5 GHz. The sample length was reduced to 5 mm at the higher frequencies to allow sufficient amplitude of the signal to be transmitted. As for the TDS measurements, pure water was used as reference. Dielectric data obtained by this method are shown in Figure 6.

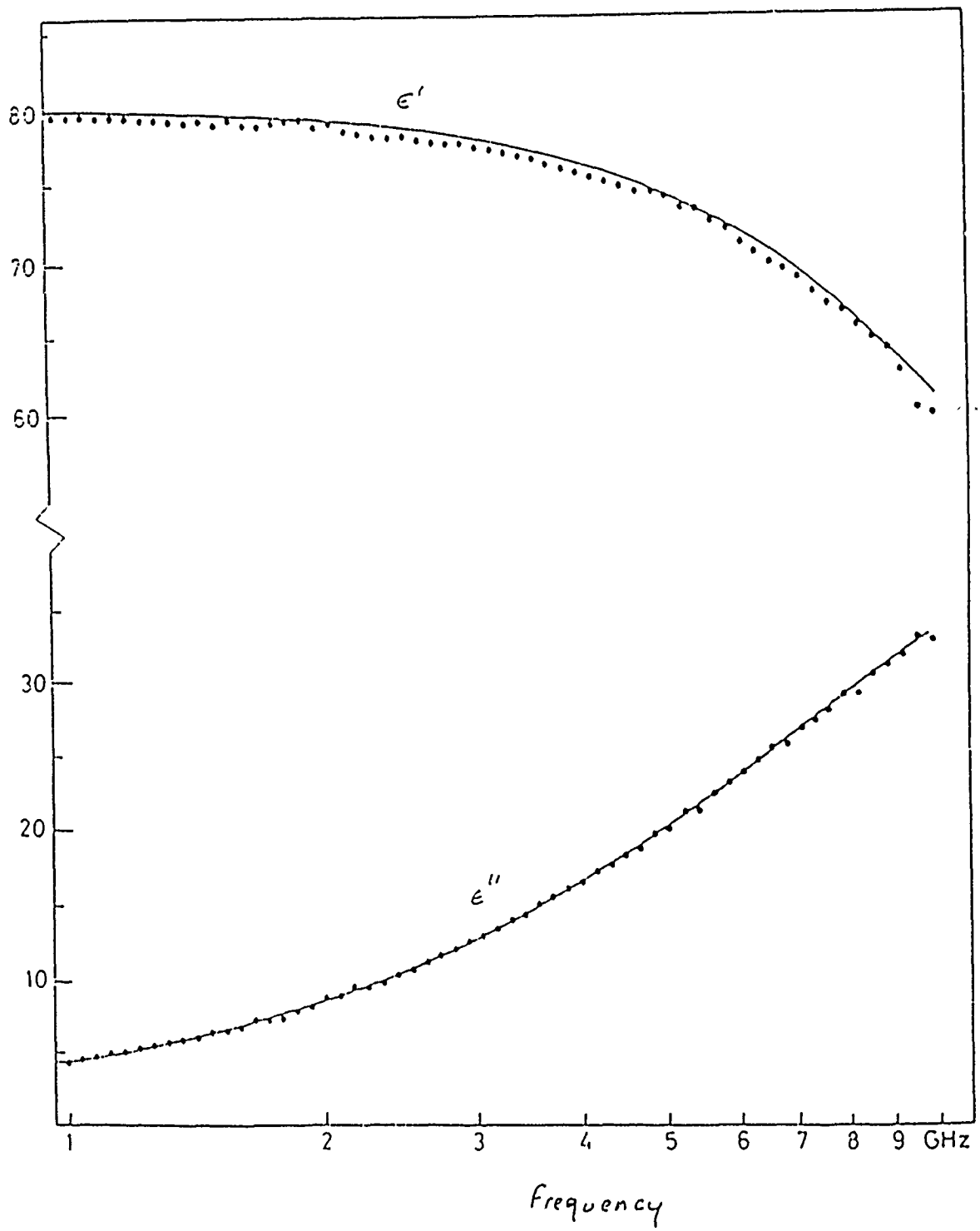
No significant differences were obtained in the measured dielectric parameters (examples of which are given in Figs.

4 - 7) for the DNA solutions, irrespective of which of the seven experimental techniques was used. The implication of the results is discussed next.

RESULTS AND DISCUSSION

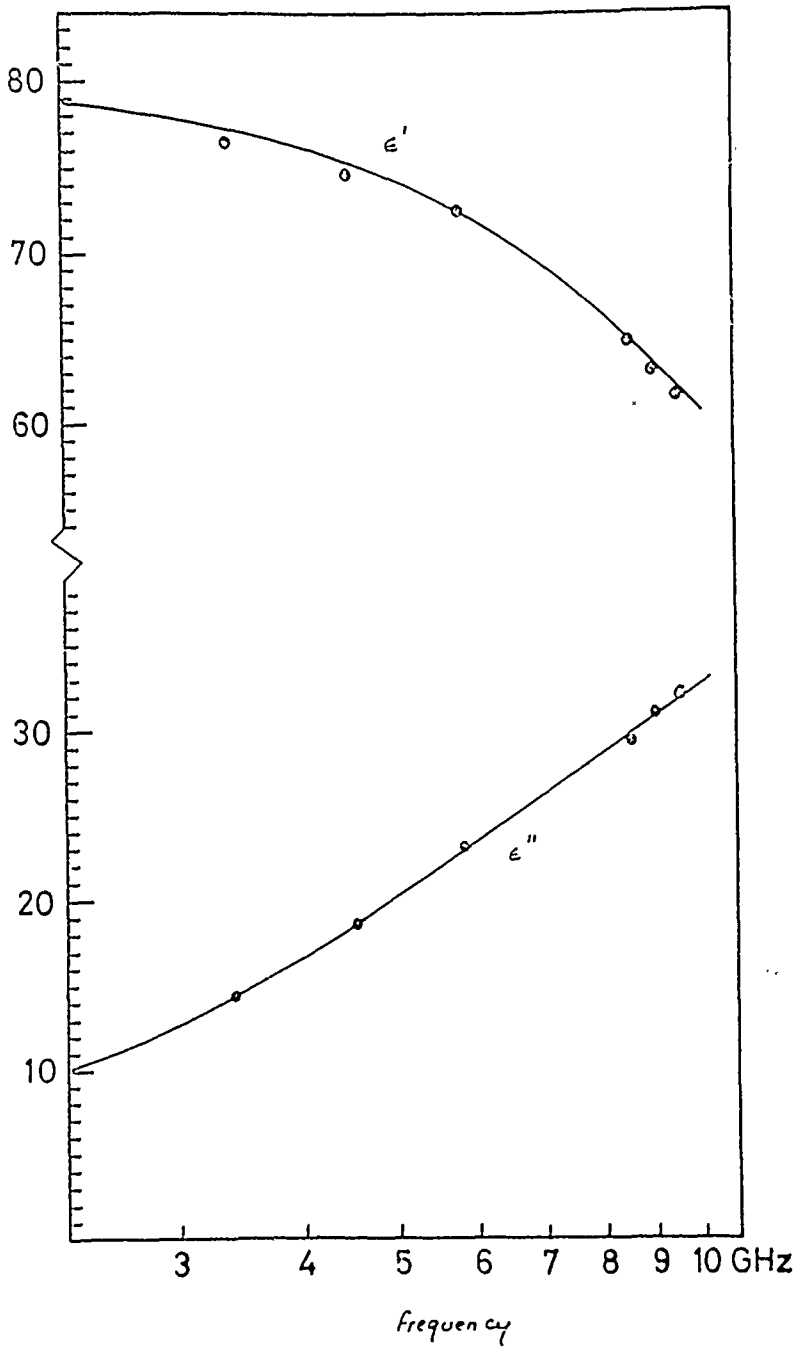
As just noted, our reason for making dielectric measurements on dilute aqueous solutions of DNA at microwave frequencies was so that a direct comparison could be made with the results obtained by Edwards et al. (8). Otherwise, little scientific interest would exist in determining the dielectric properties of an aqueous solution of concentration as low as 0.1% at frequencies in excess of 1 GHz. To obtain information of any academic value, one would have to work at a much higher concentration or, alternatively, at considerably lower frequencies where polarization mechanisms arising from the properties of the solute molecule would come into play. This subject forms the second part of the experimental research program and is introduced in the Supplement (Appendix C).

Returning to the microwave measurements, we found that *none* of the samples listed in Table 1 exhibited dielectric properties which could be distinguished from those of pure water, once corrections for ionic conductivity had been made to the dielectric loss ε''. Typical results are shown in Figures 4 and 5, where values of ε' and ε'' for supercoiled pUC8c2 are portrayed. In Figure 4 are shown the data, obtained at King's College, in which a linear scale has been chosen for frequency. This scale was chosen in order to give a direct comparison with the work of Edwards et al.(8), in which values of ε' and ε'' were displayed in the same form. The results shown in Figure 5 refer to the same samples of DNA as those in Figure 4, but were obtained in the Uppsala laboratory. In this plot, the more traditional logarithmic representation of frequency has been adopted. In both Figures 4 and 5 a continuous line is drawn to illustrate the dielectric behaviour of pure water. As would be expected in the absence of non-classical behaviour for a biological solution of concentration as low as 0.1%, there is no significant deviation from the pure water values. In both figures, each data point is the average of up to 36 independent measurements on samples from 4 plasmid preparations. In Figure 6 are shown values of ε' and ε'' for pUC8.c2 supercoiled DNA using a frequency domain method. There are fewer data points with this technique; but, nevertheless, sufficient information is in the plot to infer that the dielectric behaviour is classical. The measurements on the relaxed DNA gave the same results as those on the supercoiled form; i.e., dielectric behaviour indistinguishable from that of pure water. The experimental data are shown in Figure 7.



Literature values for pure water

Figure 5. Relative permittivity (ε') and loss factor(ε") of 0.1% plasmid (Supercoiled) DNA solution at 20 °C. (Measurement taken at Uppsala using Technique 3).



Literature values for pure water

Figure 6. Relative permittivity (ε') and loss factor(ε") of 0.1% plasmid (Supercoiled) DNA solution at 20 °C. (Measurements taken at Uppsala using measurement technique 7).

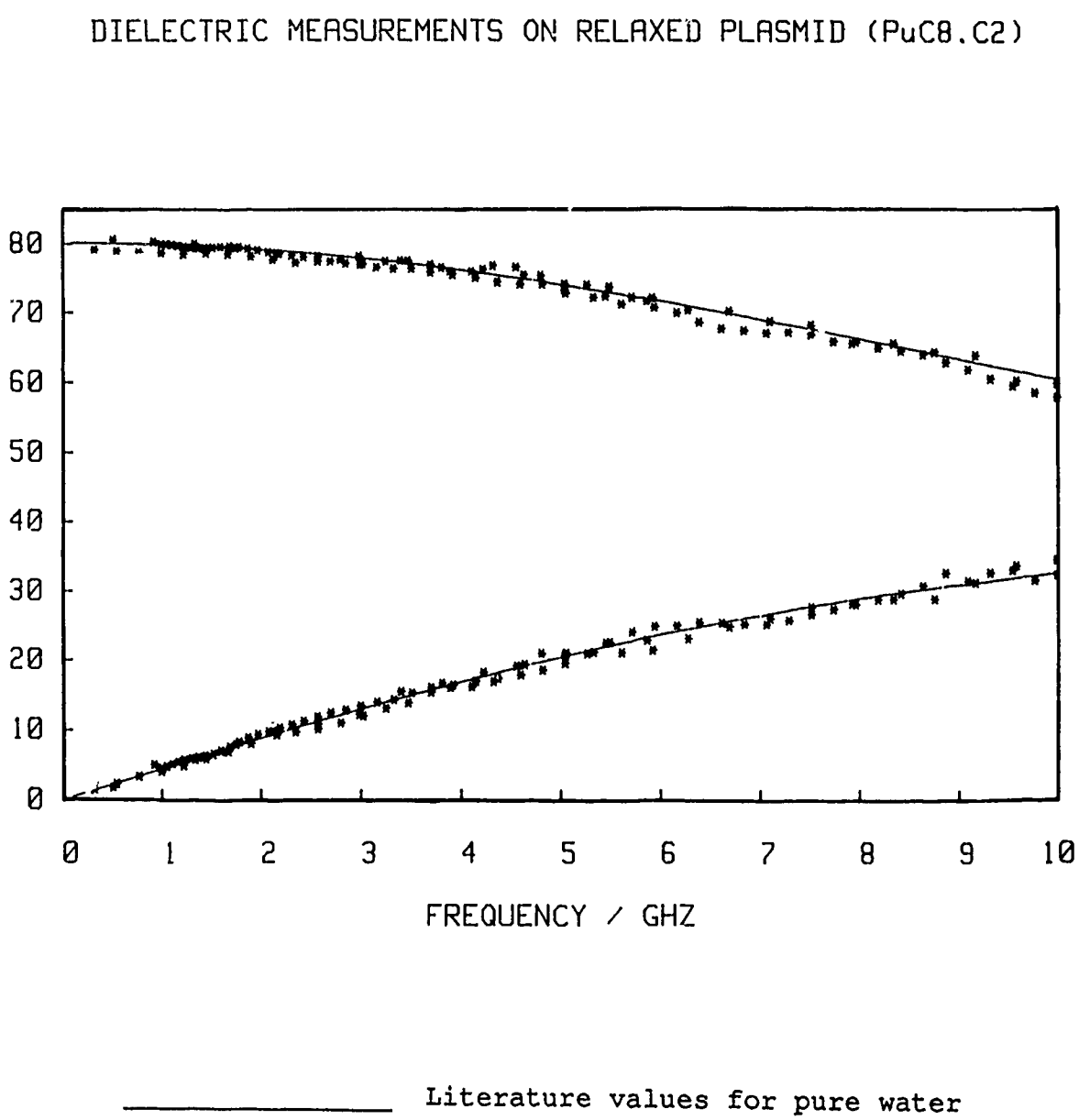


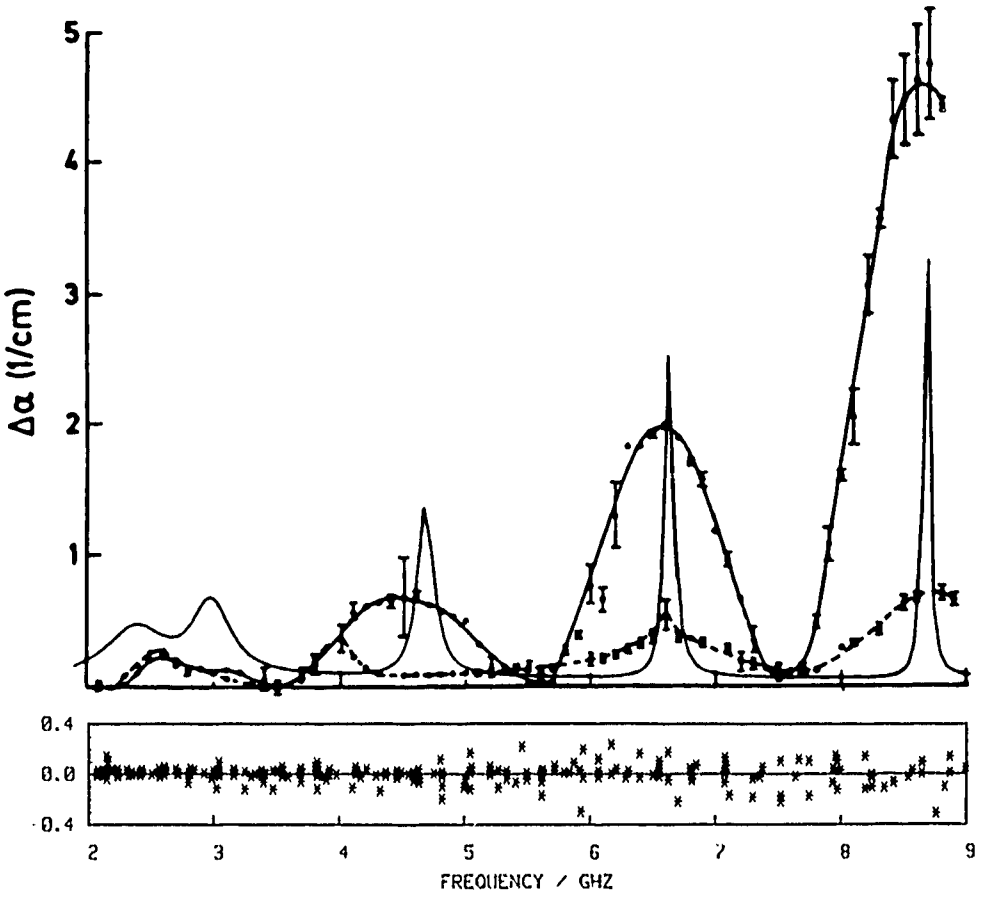
Figure 7. Relative permittivity (ε') and loss factor(ε") of 0.05% plasmid (relaxed) DNA solution at 20 °C. (Measurements taken at King's College London using measurement techniques 1 and 2).

The observation that dilute aqueous solutions of DNA might behave in a manner not consistent with the Debye or Cole-Cole dispersion equations was first made by Swicord and Davis (7), who reported an enhanced electric field attenuation coefficient (α) in the frequency region 8-12 GHz. The conclusions from this work were that there were no observed resonant peaks, the behaviour exhibited being characterised by an increase in α over the pure water value of 40% at 8 GHz, diminishing monotonically to 11% at 12 GHz. In these studies, sized fragments of *E. coli* chromosomal DNA were used -- whereas pUC8 (circular) DNA was used in their subsequent investigations (8) and in our present work (reported in this paper). The value of α may be calculated from the dielectric data, according to equation (1).

In their measurements on supercoiled pUC8 DNA, the same authors (8) displayed their data somewhat differently. In this case, for a 0.053% DNA solution, the increase in the *power* attenuation coefficient over the buffer solution was calculated and plotted as a function of frequency (Fig. 8). This change in parameter by Edwards et al. may be considered to be confusing, particularly as in the different publications they used the same symbol (α) to represent both the electric field and the power attenuation coefficients. Moreover the units are also the same. Obviously it is better <u>not</u> to mix the parameters; but, in any event, different symbols should be used (e.g.,the power coefficient might be designated α_p).

The relaxed form of pUC8.c2 DNA is of particular interest, in that the recent theoretical work of Van Zandt (22) had predicted that resonance absorption in this form of the molecule should be characterised by peaks of much greater amplitude than for the supercoiled DNA. In this same publication (22) Van Zandt showed experimental data (by Davis, Edwards, and their colleagues) on a solution of relaxed DNA, of concentration only 0.01%, which exhibited peaks in the power attenuation coefficient of magnitude greater than 30 times the value for pure water. These experimental results are shown in the upper curve of Figure 8. Finally, in the lowest part of this same figure, are shown the results obtained in the present work. In order to effect a direct comparison with the data of Edwards et al. (8,9), we have used the same form of representation; i.e., the incremental values ($\Delta\alpha_p$) of the power absorption coefficient. Note, that this method of displaying the data does not show how small a fraction of the background attenuation the amplitudes of the reported resonance peaks actually are. For example, the value of α_p for water at 2.8 GHz is 0.83 cm^{-1} rising to 7.2 cm^{-1} at 9 GHz. Thus, for the supercoiled DNA, the background value of α_p is three times the magnitude of the resonance peak Δa_p at 2.8 GHz, and 10 times the magnitude of $\Delta\alpha_p$ for the 9 GHz resonance.

All the results taken in the present work on pUC8.c1 and pUC8.c2 are included in Figure 8, relaxed and supercoiled forms



Upper Figure

——— Theoretical curve (22) based on a possible model for relaxed DNA

—•—•— Previous experimental observations (22) for 0.01% relaxed DNA

—••—••— Previous experimental observations (8) for 0.053% supercoiled DNA

Lower Figure

Present experimental values for 0.05% relaxed DNA and 0.1% supercoiled DNA. Pooled data for both types of DNA measured by the seven experimental techniques (see text).

Figure 8. Incremental power attenuation coefficient $\Delta\alpha_p$ for plasmid DNA solution at 20°C.

alike. Since it is clear from the figure that the values of the power attenuation coefficient do not deviate from those of pure water, the present results and the previous resonance data are irreconcilable. Subsequent to the commencement of the present work, it has been suggested by Foster et al.(23) that the apparently observed resonance (8) could have been caused by experimental artifacts.

CONCLUSIONS

Despite extensive measurements at microwave frequencies on three different forms of pUC8 DNA in dilute aqueous solution, no dielectric behaviour has been observed which cannot be interpreted in terms of the relaxation of water dipoles. The use of seven different experimental techniques spread over two laboratories enables this conclusion to be made strongly, notwithstanding the existence of contrary claims in the literature. A similar conclusion arises in respect to microwave measurements carried out on the effect of the enzyme DNase on calf thymus, human, and xenopus DNA. No enhanced absorption was observed, and all the data could be interpreted by the classical Debye equations for a weak electrolyte.

In view of the considerable interest aroused in these resonance phenomena, it is perhaps somewhat of an anti-climax that our findings should be wholly negative. Fortunately, the work featured in the second part of the program, which is still incomplete, is already revealing significant information concerning the interaction of radiowaves with DNA. These findings are briefly summarized in the Supplement (Appendix C), and will be reported fully next year.

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APPENDIX A

PERSONNEL INVOLVED IN THE DNA RESEARCH PROGRAM

KING'S COLLEGE LONDON

Physics Department (Dielectrics Research Group)

Professor E. H. Grant (Project Leader)
Dr. C. Gabriel
Mr. G. F. Evans
Mr. P. McArthur

Professor J. B. Bateman (Visiting Professor)

Biochemistry Department

Dr. P. R. Brown
Mrs. R. Tata

COLLABORATING RESEARCH GROUPS

University of Uppsala (Physics Department)

Dr. B. Gestblom
Dr. E. Noreland

University of Leiden (Department of Physical Chemistry, Gorlaeus Laboratories)

Professor M. Mandel
Mr. J. van der Ploeg

National Technical University of Athens (Physics Department)

Dr. A. Anagnostopoulou-Konsta

APPENDIX B

VISITS AND MEETINGS INVOLVING MEMBERS OF KING'S COLLEGE DIELECTRICS GROUP

April 1985. Dielectric properties of water in biological material. Joint Colloquium on Bioelectronics and Biosensors, 3rd University of Wales Colloquium on Biotechnology and Dielectrics Society Annual Meeting, University College of Wales, Bangor (EHG).

April-May 1985. Invited Speaker at Meeting to review Research on Biological Effects of high-Frequency Electromagnetic Fields, Asilomar Conference Center, Pacific Grove, California; organised by Science Applications International Corporation and funded by ONR (EHG).

June 1985. Comparison of the dielectric properties of normal, supercooled and frozen biological material. E. H. Grant, C. Gabriel and M. Thurai. Presented at 7th Annual BEMS Meeting. San Francisco (EHG).

June 1985. Dielectric behaviour of small biological molecules in solution. University of Lakehead, Thunder Bay, Ontario (EHG).

August 1985. Biological effects and health hazards of radiowaves and microwaves. Research Laboratories of The General Electric Company, London (EHG).

November 1985. Radiowaves and microwaves in medical and biological research. Middlesex Hospital Medical School, London (EHG).

December 1985. Interaction of radiowaves with normal and wounded human skin. E. H. Grant, C. Gabriel and R. H. C. Bentall. International Conference on Electric and Magnetic Fields in Medicine and Biology. Institution of Electrical Engineers, London (EHG and CG).

May 1986. Dielectric properties of brain and other biological material. Presentation at USAF School of Aerospace Medicine, Brooks Air Force Base, Texas, 78235 (MT and EHG).

June 1986. Dielectric properties of DNA. Department of Biomedical Electronic Engineering, University of Pennsylvania, Philadelphia (EHG).

June 1986. Dielectric relaxation in DNA solutions. C. Gabriel, P. R. Brown, R. S. Tata and E. H. Grant. 8th BEMS Annual Meeting, Wisconsin (EHG).

June 1986. Dielectric measurements on Tobacco Mosaic Virus and Tobacco Mosaic Protein J. B. Bateman, M. Thurai, C. Stevens and E. H. Grant. 8th BEMS Annual Meeting, Wisconsin (EHG).

August 1986. Visit to Dr. Bonincontro, Physics Department, University of Rome, Italy, to exchange ideas on the problems of measuring the dielectric properties of highly conducting solutions at radiofrequencies (CG).

September 1986. Biological effects of radiowaves and microwaves. Presentation at British National Committee of Electroheat Conference, St. John's College, Cambridge (EHG).

March 1987. Does microwave resonance occur in DNA? Presentation at USAF School of Aerospace Medicine, Brooks Air Force Base, Texas. Purpose of visit also to interact with scientists at Brooks to discuss matters of mutual interest (CG).

March 1987. Visit to Dr. O. Gandhi, University of Utah, Salt Lake City; to discuss low-level microwave bioeffects (CG).

June 1987. Dielectric properties of DNA solutions at radiowave and microwave frequencies, C. Gabriel, P. R. Brown, R. Tata, G. F. Evans and E. H. Grant. 9th BEMS Annual Meeting, Portland, Oregon (CG).

June 1987. Visit to Professor Bosissio, Dept. of Electrical Engineering, University of Montreal; to discuss use of 6-port hybrid reflectometer for measuring dielectric properties (CG).

June 1987. Visit to Professor G. Voss, University of Alberta; to discuss use of contact applicators for determining dielectric behaviour of lossy material (CG).

July 1987. Health hazards and medical uses of microwaves. Annual Conference of the Microwave Association. Royal Holloway College, London (EHG).

July 1987. Visit to Dr. C. Stevens, Dept. of Biological Sciences. University of Pittsburgh; to discuss use of dielectric methods for measuring hydration in biological material (JBB).

April 1988. Dielectric behaviour of water in biological material. 21st Annual Meeting of the Dielectrics Society, New College, Oxford (EHG), U.K.

June 1988 Dielectric behaviour of aqueous solutions of DNA at radiofrequencies. Presentation at University of Pennsylvania, Philadelphia (EHG).

June 1988. Dielectric properties of DNA solutions between 5 Hz - 10 MHz. C. Gabriel, R. Tata, P. R. Brown, G. F. Evans, E. H. Grant, J. van der Ploeg and M. Mandel. 10th BEMS Annual Meeting, Stamford, Connecticut (EHG).

June 1988. Visit to Dr. K. R. Foster, Dept. of Biomedical Electronic Engineering, University of Pennsylvania, Philadelphia; to discuss electrode polarization problems in relation to low frequency dielectric measuring techniques (GFE).

July 1988. Visit to Dr. R. H. Cole, Brown University, Providence, Rhode Island; to discuss time domain dielectric measurements (PMcA).

July 1988. Visit to Drs. O. P. Gandhi, C. Durney and D. Christiansen, University of Utah; to discuss modelling techniques for evaluating the fringe capacitance around a dielectric measurement probe (PMcA).

(Regular visits are made to Uppsala and Leiden for the purpose of the research collaboration).

APPENDIX C

SUPPLEMENT DESCRIBING WORK IN PROGRESS

FOR NEXT YEAR'S REPORT

Dielectric properties of DNA solutions between 5 Hz - 10 MHz over the temperature range -50 °C (-58 °F) to 20 °C (68 °F)

Prior to the present program of work, no dielectric measurements had been made on plasmid pUC8 DNA at frequencies below the microwave region. In view of the alleged resonance behaviour between 1 and 10 GHz it was clearly necessary to repair this omission, particularly as it was known from work by us and others that major dispersion regions are exhibited at radiofrequencies by large biological macromolecules. In addition to the low frequency work a program of measurements at sub-zero Celsius temperatures was also embarked on, it having been shown by us previously for ocular tissues that dielectric data on frozen material can give valuable information on the water matrix associated with the macromolecules. Both aspects of the work are incomplete; but, pending the Final Report in 1989, the current situation may be summarized briefly as follows.

Solutions of mixed plasmid (pUC8.C1 and pUC8.c2) of four differing concentrations have been measured at 20°C over the frequency range 5 Hz - 10 MHz. The measurements at the low frequency end of this range were made in the laboratory of Professor M. Mandel at the University of Leiden, Holland. The solutions exhibited two dispersion regions, corresponding respectively to counterion motion around the two different types of DNA molecule. However, the static relative permittivity was only around 130 for a solution of concentration 0.1 g/1, in sharp contrast to the known dielectric behaviour of linear DNA. This low value may be associated with the circular nature of the DNA molecule; and, to test this hypothesis, the molecule was linearised by the action of the enzyme Hind iii, and the measurements were repeated. To date, only a limited frequency range (minimum frequency 1 kHz) has been covered, but the indications are that the permittivity of the linearised molecule is indeed higher.

The measurements on the frozen samples of calf thymus DNA have indicated the presence of two dispersions in the frequency region 100 Hz - 10 MHz. The amplitude and relaxation frequency of each dispersion is dependent both on concentration and temperature. The dispersion occurring at the higher frequency, characterized by

an activation energy of around 80 kJ/mole, could be due to the same process as that observed previously in measurements by us at 20 °C (68 °F) where a relaxation frequency of 90 MHz was recorded. Complementary studies at the University of Athens using the technique of Thermally Stimulated Polarization Currents, has confirmed the existence of this dispersion, with a similar activation energy.

This brief Supplement describes work in progress pending the production, next year, of a Final Report.

U. S. GOVERNMENT PRINTING OFFICE: 1990--761-051/20036